

# **Di-Glycosyl Glyceryl Compounds for the Stabilisation and Preservation of Biomaterials**

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## DESCRIPTION

### Di-Glycosyl Glyceryl Compounds for the Stabilisation and Preservation of Biomaterials

#### FIELD OF THE INVENTION

The present invention concerns the use of di-glycosyl glyceryl compounds alone or as constituents of formulations to protect and/or stabilise enzymes or other cellular components and biomaterials against general stress, namely caused by heat, high osmolarity, free-radicals, desiccation, freeze-drying, and repetitive use.

Said compounds obey to the general formula depicted in Figure 1 and have the general name of glycosyl(1-2) glycosyl (1-2) glyceryls.

#### DESCRIPTION OF THE INVENTION AND STATE OF THE ART

The accumulation of low-molecular mass organic solutes such as, trehalose, polyols or ectoines, is a prerequisite for osmotic adjustment of many mesophilic microorganisms. However, very unusual solutes namely, di-*myo*-inositol-phosphate, di-mannosyl-di-*myo*-inositol-phosphate, diglycerol phosphate, mannosylglycerate, and mannosylglyceramide, have been identified in thermophilic and hyperthermophilic microorganisms and the intracellular content of these solutes increases in response to stress conditions, such as high osmolarity or high temperature.

Mannosylglycerate and diglycerol phosphate have been studied to a greater extent and have been shown to protect enzymes and proteins *in vitro* better than compatible solutes from mesophiles [1-3]. Moreover, the application of compatible solutes from thermophilic or hyperthermophilic organisms as stabilising agents of biomaterials has been disclosed in several patent applications [4-6].

We have discovered a novel compatible solute in the thermophilic bacterium *Picrotoga miotherma*, an organism that grows optimally at 55°C, but is

able to grow as high as 65°C. When subjected to salt stress this organism accumulates large amounts (above 1  $\mu\text{mol/mg}$  of protein) of a novel di-sugar compound. After extraction, purification, and full spectroscopic characterization by Nuclear Magnetic Resonance, we have determined the molecular structure of this, to date, unknown compound as  $\alpha(1-2)\text{mannopyranosyl-}\alpha(1-2)\text{glucopyranosyl-glycerate}$ .

It is interesting to note that the molecular structure of this compatible solute comprises the two moieties (mannosyl and glyceryl) present in mannosylglycerate, a solute widely distributed among thermophiles and hyperthermophiles [7]. In addition, there is a glucosyl moiety linking the mannosyl and glyceryl moieties.

Mannosylglycerate is a well-known biostabiliser of thermophilic origin, whose industrial application is protected under a European patent application [4]. The thermophilic origin of the novel solute combined with the structural resemblance to mannosylglycerate leads us to propose that this novel solute has stabilising properties as good or superior to those already demonstrated for mannosylglycerate. In this respect, it will serve as a stabiliser in various commercial, industrial, medical, pharmaceutical, diagnostic, cosmetic, or academic research applications.

The enhanced protein stability rendered by certain low-molecular mass organic solutes allows enzymes to function under more severe conditions of temperature, pressure, ionic strength, pH, presence of detergents or organic solvents. One of the priorities of modern biotechnology is to obtain stable enzymes or agents that stabilise those enzymes against thermal or chemical denaturation. The ability of some compatible solutes to stabilise enzymes is, therefore, of great importance to modern biotechnology. This point is obviously extended to all proteins that are used or can be used in processes where their stability is an issue, since all proteins either with or without enzymatic activity share the same overall basic elements of structure and may be protected against denaturation or inactivation through the same general mechanisms or processes.

It must also be stressed that compatible solutes protect proteins, cell membranes, liposomes, and cells from the deleterious effects of desiccation, and possess strong moistening properties. The preservation of desiccated or

lyophilized cell components and biomaterials has many applications in medicine, pharmaceutical industry, cosmetic industry, food industry, and scientific research. In spite of the great importance of desiccation and freezing in the conservation of biological samples, denaturation of proteins or a decrease of the viable count of cultures inevitably takes place during utilization, and could be prevented or diminished by the use of low molecular mass stabilisers.

Also, the stability of nucleic acid molecules, like DNA, or RNA, can be improved by the addition of compatible solutes from hyperthermophiles, as described for ectoines [8], and their use in several applications in medicine, pharmaceutical industry, or scientific research can be envisioned.

## BRIEF DESCRIPTION OF THE DRAWING

### Figure 1.

Depicts the generic chemical structure of glycosyl(1-2)glycosyl(1-2)glyceryls in all its possible stereoisomeric forms. The figure is intended to represent all hexoses either in the  $\alpha$  or in the  $\beta$  configuration. The letter "R" is intended to represent a carboxylate, an amide, a primary alcohol group, or a methyl group.

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